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Summary of Workshop on Automation

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A workshop was held to discuss the value, implication and implementation of automation techniques for pollen analysis in the specific case of biological activity detection.

To initiate the discussion, three questions were presented to the discussants:

- Is there a need for automation?
- How well is the biology worked out?
- What is the data desired?

The results of an informal vote were the nearly unanimous position that there was a need for automation. Discussion of this surprising result indicated the strong feeling that substantial time and effort was spent in analyzing pollen by hand and that this could be done by machines. A further viewpoint expressed was the desirability of using the increased sensitivity and objectivity to score particles for the degree of biological activity.

In the course of discussion, four specific objectives were determined to be desirable for machine implementation: (a) obtain a count of the number of mutant pollen grains; (b) obtain a count of the number of total grains in a sample; (c) obtain a count of the number of viable pollen grains; (d) obtain a count of the number of non-pollen particles (debris). Requirement (b) is recognized as the denominator of both the mutant fraction [requirement (a) divided by requirement (b)] and the viable fraction [requirement (c) divided by requirement (b)] . Requirement (d) was recognized as not essential for the human analysis but with machine analysis discrimination of the debris or non-pollen particulates in the sample may be required. An additional aspect of instrumented analysis is its ability to provide new information by either increased sensitivity, resolution or objectivity. In particular, a desire was expressed for the need to measure accurately the hue and intensity of the iodine uptake by mutant pollen grains. It was felt that a measure of the degree of biological response could be elucidated by such measurements.

In spite of the fact that a need for automation was expressed by the discussants, no consensus was arrived at the form that this automation should take. For example, the enumeration of the frequency of mutant events or viable events is suitably performed on flow systems; on the other hand, the determination of an objective measure of individual pollen grains is more suitably determined by high resolution imaging systems. The standard engineering problems in automation, the cleanliness of the sample and the reliability of the staining system, were discussed and recognized as obstacles to be overcome in the specific implementation of the automation technology.

In biological assay systems, automation usually deals with the counting problem. The usefulness of such automation is compromised if counting is not a limitation that can be considerably reduced by these techniques; in such a case, effort to reduce the time for the assay needs to go elsewhere. Therefore, a poll was taken of the amount of time required to perform the analytical portion of the assay. Two separate counting methodologies were presented, one dealing with an estimate of the total number of pollen grains and the other one in which the total number of pollen grains were actually counted. To obtain a count of 1 million pollen grains, the times varied from a low of ½ hr to a typical period of 7–8 hr to a high of 35 hr. The lower figures were obtained from groups whose laboratories estimate the total number of pollen grains, whereas the high figure was obtained from groups whose laboratories obtain a direct count of the grains. The laboratories reporting the low time figure require 20-fold repetition of the counts for reliable numbers which results in a sample count on the order of several hours. Estimation techniques can be used to determine the denominator (see above), because it is so large it can tolerate a

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substantial error; for the numerator (for example, the number of mutant grains) a relatively small error can be catastrophic.

As a personal note of conclusion from this writer, this workshop served an excellent function to orient engineers or individuals concerned with the engineering function of automated methodology to the needs and aspirations of individuals who are actively involved in determination of pollen grain mutations. The workshop dealt primarily with analysis of ovoid pollen particles. There was insufficient time to cover in detail other interesting problems in enumeration of pollen behavior (e.g., determination of pollen tube length). It is quite clear from the workshop that a substantial number of investigators would like to minimize if not eliminate the drudgery of obtaining mutant frequency or viable pollen frequency. Clearly, substantial individualism was expressed by the participants in assessing the need for automation in their laboratories which was directed toward the particular type of experiments that they were performing. Such a diversity of opinion is, of course, to be encouraged, since the need for automation must be based on the needs of the laboratory rather than vice versa.